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Cobitis beijingensis, a new spined loach from northern China (Cypriniformes, Cobitidae)

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Abstract

A new spined loach, *Cobitis beijingensis*, is described from Beijing, China. The new species can be distinguished from other congeners by a combination of characters: 1) Lamina circularis on pectoral fin of male elongated, posterior margin slightly serrated; 2) possessing 14–18 (mode 15) pre-pelvic myotomes; 3) pelvic fin inserted below 3rd–5th branched dorsal-fin ray; 4) total vertebrae 4+ 36–38 (mode 37); 5) L_2 of the Gambetta's pattern does not exist or is fused with L_1. The molecular phylogenetic analyses based on mitochondrial Cyt *b* also support *C. beijingensis* to be a new species.

Key Words

Beijing, freshwater fish, molecular phylogeny, morphology, taxonomy

Introduction

The genus *Cobitis* Linnaeus, 1758, is a widespread and species-rich group of small freshwater fishes that have a slender body and are adapted to the benthic habitat (Chen and Chen 2013). It can be found in most of the river systems from Europe and Northern Africa to Asia (Fricke et al. 2024). This group of small freshwater fishes possesses lamina circularis on pectoral fins in males, bifurcated suborbital spines, and five longitudinal lines along the body (Kim 2009). To date, over a hundred valid species have been identified, and the discovery of new species continues (Nakajima and Suzawa 2016; Nakajima 2016; Chen et al. 2018; Freyhof et al. 2018; Vasil'eva et al. 2020; Froese and Pauly 2024). China, one of the hotspots of the *Cobitis* species diversity, has approximately twenty-three valid species (Chen et al. 2015, 2016, 2018; Chen and Chen 2016), and the majority of the species are distributed in southern China, except for five valid species discovered in northern China: *Cobitis melanoleuca* Nichols, 1926; *C. granoei* Rendahl, 1935; *C. lutheri* Rendahl, 1935; *C. choii* Kim & Son, 1984; and *C. gracilis* Chen & Chen, 2016.

Beijing, located in the Haihe River basin of northern China, is one of the distribution areas of *Cobitis* species (Chen and Chen 2016). Zhang and Zhao (2013) noted that the body shape and color pattern variant in *Cobitis* spp. from Beijing might represent different species. In our most recent expeditions and subsequent examination of historical specimens at the Institute of Zoology, Chinese Academy of Sciences, we noticed that Beijing is home to at least two species of *Cobitis* fishes: *C. melanoleuca* Nichols, 1925, and an as-yet undescribed species. In this study, we present a detailed description of the new species.

Materials and methods

Specimen collection, examination, and preservation

All examined specimens (n = 118) were collected by hand net, fish trap, cast net, or bought from the local markets. Detailed information on the specimens is listed in the Comparative Materials. Specimens used for

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morphological study were initially fixed in 10% formalin solution for three days, followed by 70% ethanol alcohol for long-term preservation. Specimens used for molecular phylogenetic study were fixed in 95% ethanol alcohol directly. The holotype and paratypes of the new species were deposited at the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (**ASIZB**; abbreviation follows Leviton et al. 1985). Other examined materials were deposited at ASIZB and the Museum of Comparative Zoology, Harvard University (**MCZ**). The historical specimens from Fan Memorial Institute of Biology (**FMZB**) were also included in the examination and are now deposited at ASIZB.

Morphological study

All measurements were taken point to point with digital calipers to 0.01mm on the left side of the specimens. Counts were also made on the left side of specimens. The specimens (n = 19) used for the vertebral count were radiographed with an XDR-AZ1600 X-ray machine produced by Shanghai Anzhu Photoelectric Technology Co., LTD. X-ray tube voltage 60 kV and the tube current 1 mA. Exposure time: 2s. Standardized measurements, landmarks and meristic counts for the genus *Cobitis* were listed in detail to build a series for this study.

Counts

Dorsal, anal, pectoral, pelvic-fin rays: the number of unbranched rays and branched rays of fin. The last one and half branched fin rays of dorsal and anal fins are usually counted as one. Gambetta's pattern: The longitudinal rows of pigmentation along the body. The first Gambetta line (L_1) is located on the dorsal; the second to fifth Gambetta lines (L_2–L_5) are located on the lateral side sequentially (Gambetta 1934). Shape and number of the blotches in each Gambetta line are counted and documented. Notations (L_1–L_5) describing the Gambetta's pattern followed Takeda and Fujie (1945). Prepelvic myotome counts: The number of segmented muscle plates between the base of the pectoral fin and the insertion of the pelvic fin (Nakajima and Suzawa 2016). Vertebral counts: The Weberian apparatus was counted as four vertebrae. The last half centrum was counted as one vertebra.

Body morphometric measurements

The specific morphometric measurements of the body are shown in Fig. 1. Standard length (SL, A–B), from tip of the snout to the last half-centrum; body depth (C–D) from the insertion of the dorsal fin vertically to the ventral midline; head length (HL, A–E), from tip of the snout to the most posterior point of the operculum; head depth (F–G),

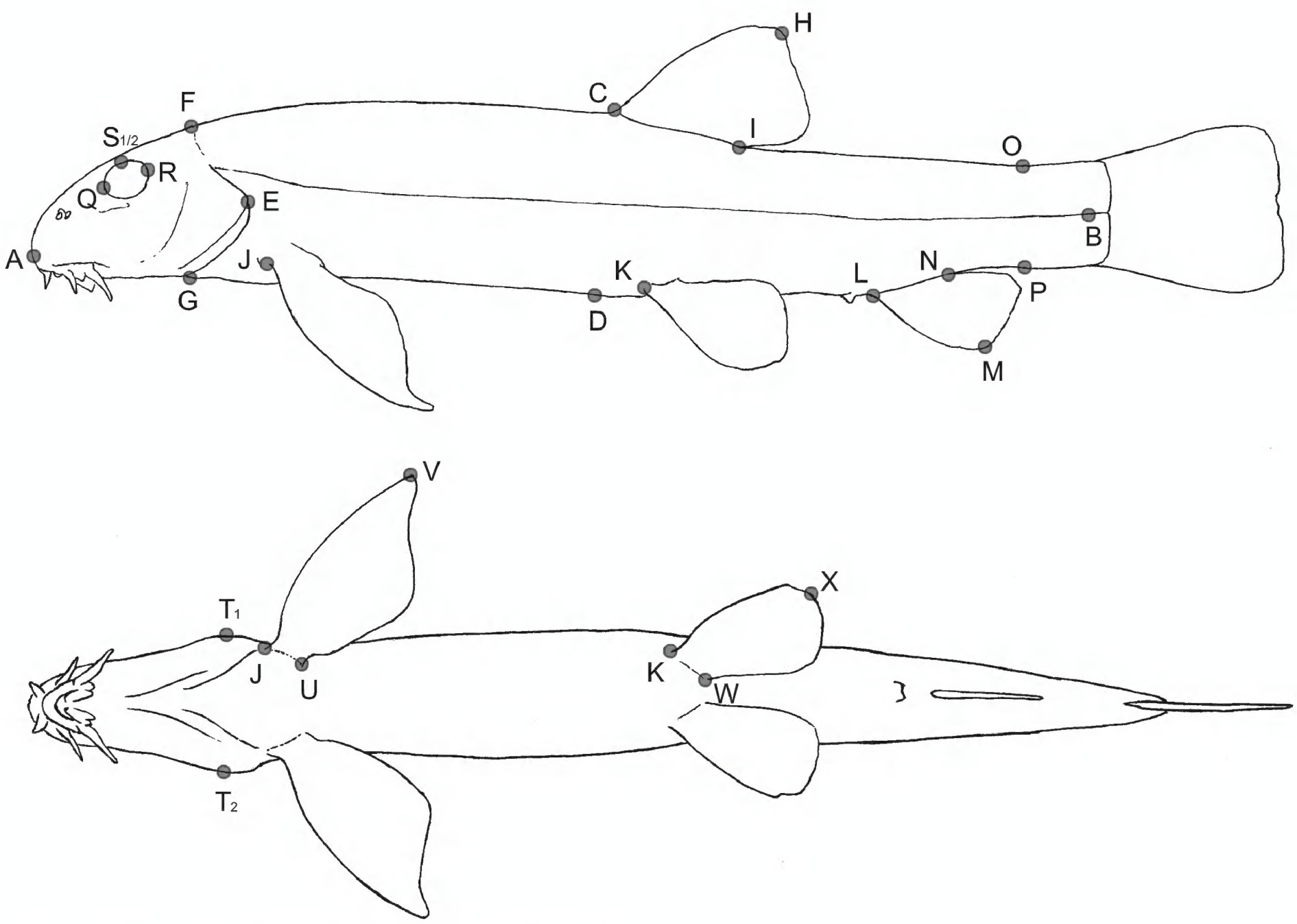

Figure 1. Illustration of morphometric measurements for *Cobitis* species.

from the position near the occiput vertically to the venter; head width (T_1–T_2), distance between operculum on both sides; pre-dorsal length (A–C), from the tip of the snout to the dorsal fin insertion; post-dorsal length (C–B), from the dorsal-fin insertion to the last half-centrum; pre-anal length (A–L), from the tip of the snout to the anal-fin insertion; pre-pectoral length (A–J), from the tip of the snout to the pectoral-fin insertion; pre-pelvic length (A–K), from the tip of the snout to the pelvic-fin insertion; caudal peduncle length (N–B), from the end of anal-fin base to the last half-centrum; caudal peduncle depth (O–P), the minimum vertical distance from the dorsal side to the ventral side of the caudal peduncle; dorsal-fin length (C–H), from the dorsal-fin insertion to the end of the longest dorsal-fin ray; dorsal-fin base length (C–I), from the dorsal-fin insertion to the end of the dorsal-fin base; anal-fin length (L–M), from the anal-fin insertion to the end of the longest anal-fin ray; anal-fin base length (L–N), from the anal-fin insertion to the end of the anal-fin base; pectoral-fin length (J–V), from the pectoral-fin insertion to the end of the longest pectoral-fin ray; pectoral-fin base length (J–U), from the pectoral-fin insertion to the end of the pectoral-fin base; pelvic-fin length (K–X), from the pelvic-fin insertion to the end of the longest pelvic-fin ray; pelvic-fin base length (K–W), from the pelvic-fin insertion to the end of the pelvic-fin base.

Molecular phylogenetic analyses

The molecular study was based on the mitochondrial cytochrome-*b* (cyt-*b*) sequences (1140 bp). DNA was extracted from the pelvic fin on the right side of the loach. Cyt-*b* was amplified using the universal primers L14724 (5'-GACTTGAAAAACCACCGTTG-3') and H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3'). Sequencing reactions were performed according to the operating instructions of BigDye Terminator v3.1 (BDT), with 1μL of primer (3.2 pmol/μL), 1 μL of template DNA, 2 μL of BigDye® Terminator v3.1, and 6 μL of double distilled water (dd H2O) for a total reaction volume of 10 μL. The thermo-cycling conditions were: initial denaturation for 2 min at 96 °C, denaturation for 10 s at 96 °C, annealing for 10 s at 50 °C, and extension for 1 min at 60 °C. After 30 cycles, a final extension was performed at 60 °C for 3 min, and the polymerase chain reaction (PCR) products were preserved at 4 °C. Sequencing was conducted by Beijing TsingKe Biotech Co., Ltd. (China).

The sequencing results were assembled using SeqMan II; other sequences were acquired from the NCBI (https://www.ncbi.nlm.nih.gov/). The voucher ID of each sequence and GenBank accession number are given in Table 1. Thirty Cyt-*b* sequences of *Cobitis* species were included in the molecular phylogenetic analyses. *Sabanejewia aurata* was used as an outgroup. Nucleotide sequence alignment was conducted using MEGA v6.0 (Tamura et al. 2013) with ClustalW.

ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit model using Bayesian information criterion (BIC). The Bayesian inference (BI) phylogenies were inferred by MrBayes v3.2.6 (Ronquist et al. 2012) under the HKY+F+G4 model (two parallel runs, 1 000 000 generations), and the initial 25% of sampled data was discarded as burn-in. The best-fit model for maximum likelihood (ML) phylogenetic analysis was selected using MEGA 6.0, and the ML tree was also reconstructed in the same software under the HKY+G model it selected (10 000 bootstrapping replications). Trees were visualized by TVBOT (https://www.chiplot.online/tvbot.html, Xie et al. 2023). Haplotypes were detected by DnaSP v.5.10.01. Each sequence was labeled with the taxonomic nomenclature, and then the evolutionary divergence of sequence pairs between and within groups (i.e., species) was estimated using the Kimura 2-parameter model (Kimura 1980).

Results

***Cobitis beijingensis* Sun & Zhao, sp. nov.**

https://zoobank.org/E36C5C10-3A0F-4675-9E1C-89039B229C07

Figs 2–4; Table 2

Type material. ***Holotype.*** • ASIZB 240630, male, 55.0 mm SL; Shilipu Township, Miyun District, Beijing, from the Chaobaihe River (40.33574769°N, 116.79236588°E, 52 m a. s. l.); collected by Zhixian Sun, Junyuan Hao and Dong Sheng; 13 June 2024.

Paratypes. Twenty-five specimens • ASIZB 240631–6, 240689–95, 3 males, 10 females, 50.2–75.3 mm SL; same collection information as holotype • ASIZB 240697–8, 2 juveniles, 24.1–27.0 mm SL; same locality and collectors as holotype; 14 June 2024 • ASIZB 240682–4, 3 males, 48.8–58.1 mm SL • ASIZB 240685–8, 4 juveniles, 26.4–33.1 mm SL; same locality as holotype; collected by Zhixian Sun and Wenli Yu; 15 June 2024 • ASIZB 240605, 240680–1, 1 male, 2 females, 56.1–74.5 mm SL; Qiaozi Township, Huairou District, Beijing, from the confluence of the Huaijiuhe River and Huairou Reservoir (40.31521727°N, 116.57391776°E, 64 m a. s. l.); collected by Zhixian Sun, Yutian Fang and Dong Sheng; 10 May 2024.

Additional materials examined. Twenty-five specimens • ASIZB 62425–8, 1 male, 3 females, 42.0–67.0 mm SL; Niulanshan Township, Shunyi District, Beijing, from the Chaobaihe River; collected by Chunguang Zhang; 1992 • ASIZB 192934 (FMZB 3861), female, 53.0 mm SL; Shahe Township, Changping District, Beijing; 1 June 1929 • ASIZB 16918, female, 92.2 mm SL; Beijing • ASIZB 17732, female, 75.8 mm SL; Beijing • ASIZB 17388 (FMZB 6030), 17390 (6032), from one lot of specimens (ASIZB 17388–99), 1 male, 1 female, 51.4–54.0 mm SL; Changxindian Township (originally in Hebei Province), Fengtai District, Beijing, from the Yongdinghe River; collected by Yingxiang Du; 9 May 1930 • ASIZB 17297–802, gender unidentified, 6 specimens, 59.4–68.5 mm SL; Liulihe Township, Fangshan District, Beijing, from the Liulihe River • ASIZB 103008–10, 2 males, 1 female, 50.5–65.7 mm SL; Anxin County, Baoding City,

Table 1. Voucher codes, sampling localities, haplotypes, and accession numbers of *Cobitis* species and outgroup for molecular analyses.

Voucher Code	Species	Locality	Drainage	Haplotype	Accession no.	Source
ASIZB 240680	*C. beijingensis* sp. nov.	Huairou District, Beijing, China	Haihe River basin	H1	–	This study
ASIZB 240681	*C. beijingensis* sp. nov.	Huairou District, Beijing, China	Haihe River basin	H2	–	This study
ASIZB 240690	*C. beijingensis* sp. nov.	Miyun District, Beijing, China	Haihe River basin	H3	–	This study
ASIZB 240692	*C. beijingensis* sp. nov.	Miyun District, Beijing, China	Haihe River basin	H3	–	This study
ASIZB 240693	*C. beijingensis* sp. nov.	Miyun District, Beijing, China	Haihe River basin	H3	–	This study
ASIZB 240697	*C. beijingensis* sp. nov.	Miyun District, Beijing, China	Haihe River basin	H3	–	This study
ASIZB 240698	*C. beijingensis* sp. nov.	Miyun District, Beijing, China	Haihe River basin	H4	–	This study
ASIZB 238671	*C. lutheri*	Helong City, Jilin Province, China	Tumen River basin	H5	–	This study
ASIZB 240671	*C. lutheri*	Wangqing County, Jilin Province, China	Suifen River basin	H6	–	This study
ASIZB 240673	*C. lutheri*	Wangqing County, Jilin Province, China	Suifen River basin	H7	–	This study
ASIZB 240675	*C. lutheri*	Wangqing County, Jilin Province, China	Suifen River basin	H8	–	This study
ZMMU P-21919	*C. lutheri*	Primorye District, Russia	Tumen River basin	H9	JN858891	Perdices et al. 2012
–	*C. lutheri*	Primorye District, Russia	Heilongjiang River basin	H10	JN858892	Perdices et al. 2012
ZMMU P-21935	*C. lutheri*	Khabarovsk District, Russia	Heilongjiang River basin	H11	JN858893	Perdices et al. 2012
–	*C. lutheri*	Linjiang City, Liaoning Province, China	Yalu River basin	H12	KY009953	Chen and Chen 2016
–	*C. hankugensis*	Korea	–	H13	MN841275	Alam et al. 2019 (Direct Submission)
–	*C. hankugensis*	Hapcheon, Gyeongsangnam-do, Korea	Nakdong River basin	H13	MZ339224	Lee et al. 2022
–	*C. nalbanti*	Gongju, Chungcheongnam-do, Korea	Geum River basin	H14	KF661552	Kwan et al. 2013
–	*C. nalbanti*	Jeonju, Jeollabuk-do, Korea	Mangyeong River basin	H15	KF661686	Kwan et al. 2013
–	*C. tetralineata*	Bokeung, Jeollabuk-do, Korea	Dongjin River basin	H16	KF661612	Kwan et al. 2013
–	*C. tetralineata*	Imsil, Jeollabuk-do, Korea	Dongjin River basin	H16	KF661717	Kwan et al. 2013
–	*C. tetralineata*	Gokseong, Jeollanam-do, Korea	Boseong River	H16	KC524528	–
ASIZB 231802	*C. melanoleuca*	Huairou District, Beijing, China	Haihe River basin	H17	–	This study
ASIZB 231807	*C. melanoleuca*	Huairou District, Beijing, China	Haihe River basin	H18	–	This study
–	*C. granoei*	Melnikovo, Tomsk District, Russia	Ob River basin	H19	KM583513	Perdices et al. 2014
–	*C. granoei*	Zhigalovo, Irkutsk District, Russia	Lena River basin	H20	KM583532	Perdices et al. 2014
ASIZB 235773	*C. gracilis*	Ji'an City, Jilin Province, China	Yalu River basin	H21	–	This study
–	*C. gracilis*	Linjiang City, Liaoning Province, China	Yalu River basin	H22	KY009955	Chen and Chen 2016
ASIZB 221108	*C. choii*	Xinglong County, Hebei Province, China	Luanhe River basin	H23	–	This study
–	*C. choii*	Yocheon, Jeollabuk-do, Korea	Geum River basin	H24	EF508510	Šlechtová et al. 2008
–	*Sabanejewia aurata*	Georgia	Rioni River	–	AF499190	Perdices et al. 2003

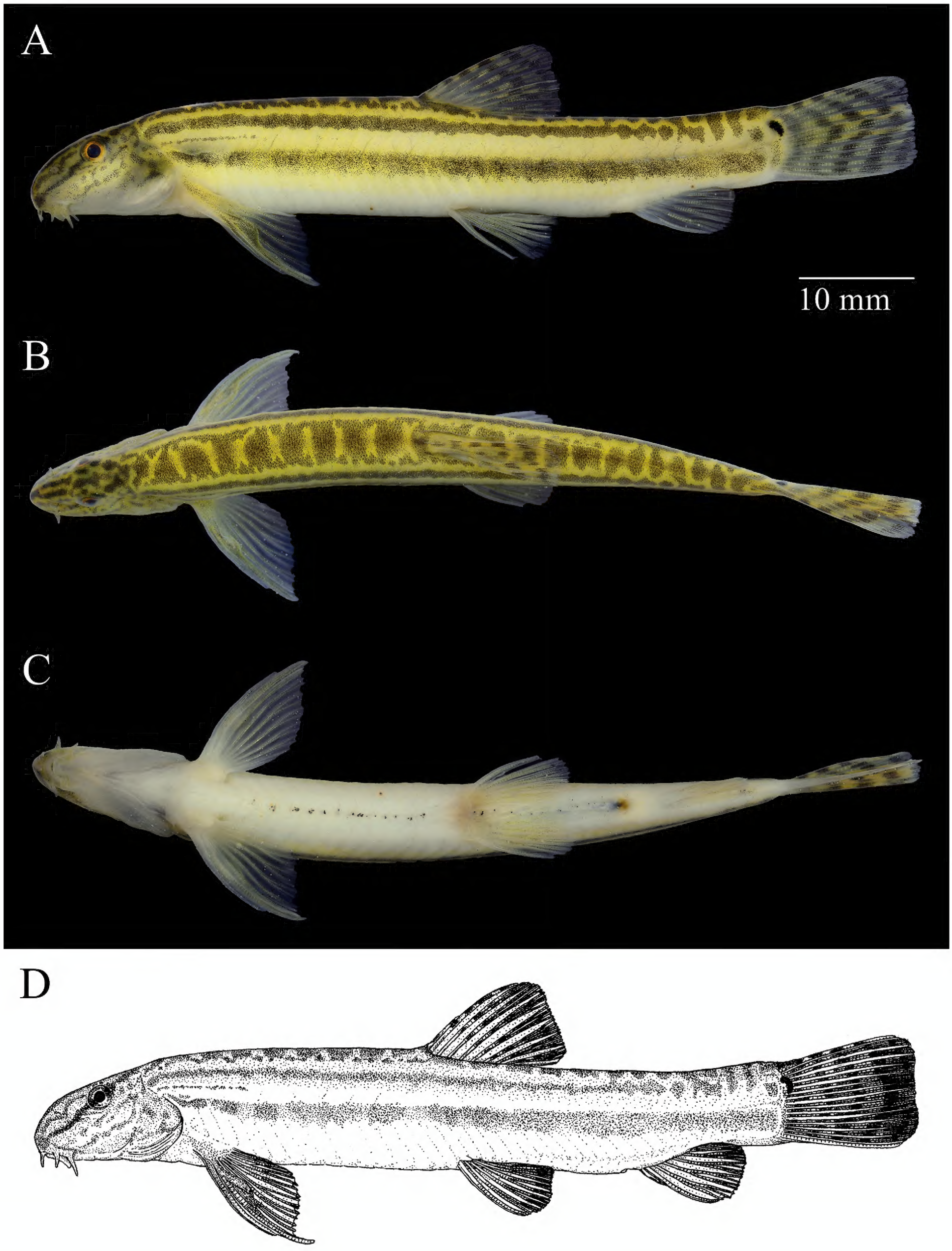

Figure 2. *Cobitis beijingensis* sp. nov., holotype, ASZIB 240630, 55.0 mm standard length; **A.** Lateral view; **B.** Dorsal view; **C.** Ventral view; **D.** Original drawing.

Hebei Province, from the Baiyangdian Lake system; collected by Shiyi Zhang and Wenbin Wang; October 1959 • ASIZB 16917, female, 116.3 mm SL; Xinji Township, Sanhe City, Langfang City, Hebei Province, from the Juhe River; 13 June 1930 • ASIZB 79421–5, 5 females, 58.3–109.4 mm SL; Anxin County, Baoding City, Hebei Province, from the Baiyangdian Lake system • ASIZB 170316, female, 62.4 mm SL; Xisiduhe Village, Jiuduhe Township, Huairou District, Beijing, from the Huaijiuhe River; 1 June 1974 • ASIZB 240696, male, 54.5 mm SL; Lvhu local market, Qiaozi Township, Huairou District, Beijing, from the Huairou Reservoir; collected by Zhixian Sun, Junyuan Hao and Dong Sheng; 15 June 2024.

Diagnosis. The new species can be distinguished by the combination of the following characters: Lamina circularis on pectoral fin elongated, posterior margin slightly serrated; 14–18 (mode 15) prepelvic myotomes; pelvic fin inserted below 3rd–5th branched dorsal-fin ray; total vertebrae 4+ 36–38 (mode 37); the second Gambetta line (L_2) does not exist or is fused with the first line (L_1).

Description. Body elongated, laterally compressed. Dorsal body profile rising smoothly from nostrils to occiput, almost horizontal between occiput and dorsal-fin insertion, gradually sloping along dorsal-fin base, then horizontally extending to caudal-fin base. Maximum body depth at dorsal-fin origin, body depth 12.6–19.8% of standard length. Lower surface of head flattened, abdomen rounded. Caudal peduncle short, depth almost equal to length, laterally compressed, with fleshy keels on dorsal and ventral sides. Head length greater than body depth (101.0–156.7% of head length); snout elongated and blunt, snout length 36.9–50.1% of head length; eye small, 11.2–20.3% of head length, superior and laterally positioned, near gill opening than tip of snout; nostrils positioned close together, anterior nostril with short tube; interorbital region ridged and narrow. Anus positioned near anal-fin insertion.

Mouth inferior, lips fleshy. Upper lip without affiliated apparatus; lower lip two lobed, medial side elongate-oval shaped. Three pairs of barbels: rostral barbels shorter than maxillary and mandibular barbels; maxillary barbels length equal to mandibular barbels length; mandibular barbels not or almost reaching under the anterior edge of the orbit.

Body covered with tiny cycloid scales. Prepelvic myotomes 14 (3 specimen), 15 (26), 16(7), 17(2), 18(1).

Dorsal fin with three unbranched and six (1) or seven (39) branched rays; distal margin slightly convex, inserted nearer to caudal-fin base than snout. Pectoral fin with one unbranched and seven (3), eight (32) or nine (5) branched rays. Pelvic fin with one unbranched and five (1), six (38) or seven (1) branched rays, inserted below third to fifth branched dorsal-fin ray. Anal fin with 3 unbranched and 5 (40) branched rays; inserted nearer to caudal-fin base than to pelvic-fin insertion. Caudal fin slightly truncated, with one unbranched principle ray and seven branched principle rays on upper part and seven branched principle rays and one unbranched principal ray on lower part.

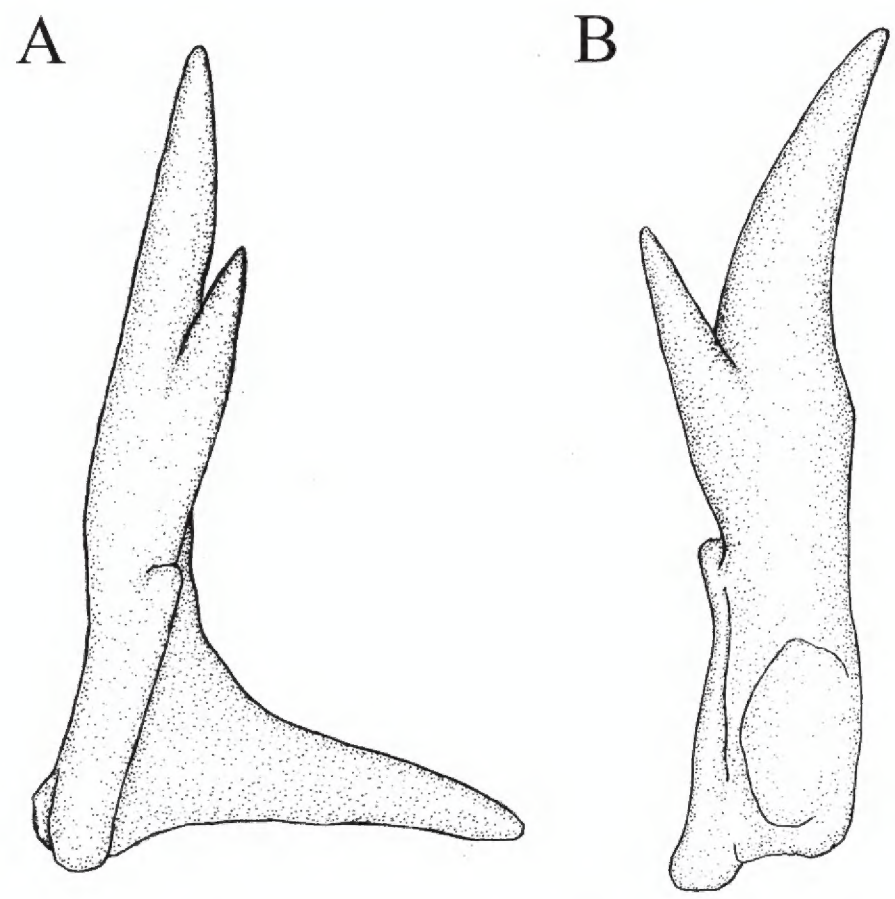

Figure 3. Right suborbital spine of *Cobitis beijingensis* sp. nov. **A.** Lateral view; **B.** Dorsal view.

Total vertebrae 4+36 (1), 37 (6), 38 (4). Suborbital spine bifurcated (Fig. 3), posteriorly reaching under the center of the orbit.

Coloration in live. Dorsal side of head and body yellowish brown, mid-lateral side light yellowish brown, ventral side grayish white. A clear black stripe or "tear mark" runs from tip of snout, crossing the eye, to the occiput; two dark grey stripes distributed parallel to the clear black stripe, one on the dorsal side of the head; one runs across the nostrils; several vague and irregular patterns located under the eye and opercula, sometimes forming one or two grey stripes parallel to the clear black stripe. Four obvious lines of Gambetta's pattern. The L_1 of Gambetta's pattern on the dorsal of body, 7–10 black blotches before dorsal-fin insertion, 7–11 black blotches after dorsal-fin base end; L_2 not exist or fused with L_1; L_3 linear, interrupted into several small irregular blotches and fused with L_1 at caudal peduncle; L_4 linear, consisted of many tiny spots; L_5 obvious, extending along the mid-lateral side of body, consisted of 9–15 different sized black blotches, sometimes connected with each other with black strip. Three or four rows of pigmentation on dorsal-fin rays; three or four arcuate black bars on caudal fin. One big jet-black oval-shaped spot on upper caudal-fin base, size comparable to the eye diameter (Fig. 4).

Coloration in preservation. Dorsal side of head and body yellow, mid-lateral side light yellow, ventral side pale. All patterns same position as living specimen.

Sexual dimorphism. Males with elongated lamina circularis derived from the base of the first branched pectoral-fin ray; tip of the lamina circularis almost reaching the mid-point of the first branched ray; posterior margin of the lamina circularis usually serrated (Fig. 5A). The L_4 of Gambetta's pattern in males, usually thin and short, sometimes disappeared. The blotches on L_5 in males usu-

Figure 4. Live individuals of *Cobitis beijingensis* sp. nov. **A.** Holotype, ASIZB 240630, male; **B.** Uncatalogued, female; **C.** Paratype, ASIZB 240682, male, photographed in aquarium.

ally merged into a line. Females usually larger and slender than males. The L_4 in females usually exceeds over dorsal fin insertion, sometimes reaching the caudal peduncle. The blotches on L_5 in females not fully merged into one line.

Distribution. Based on our field collections and the historic specimens, *Cobitis beijingensis* is distributed in the Haihe River basin in northern China, including rivers in Beijing and the Baiyangdian Lake in Hebei Province. This species can be currently found in the Chaobaihe River system in Huairou and Miyun District, Beijing (Fig. 6).

Habitat and biology. This species usually inhabits slow-flowing water or still water with plenty of submerged plants or filamentous algae. The benthic microhabitat is usually sand and silt mixed with some gravel. Coexisting species include *Rhodeus ocellatus*, *Carassius auratus*, *Misgurnus anguillicaudatus*, *Oryzias latipes*, *Rhinogobius giurinus*, etc.

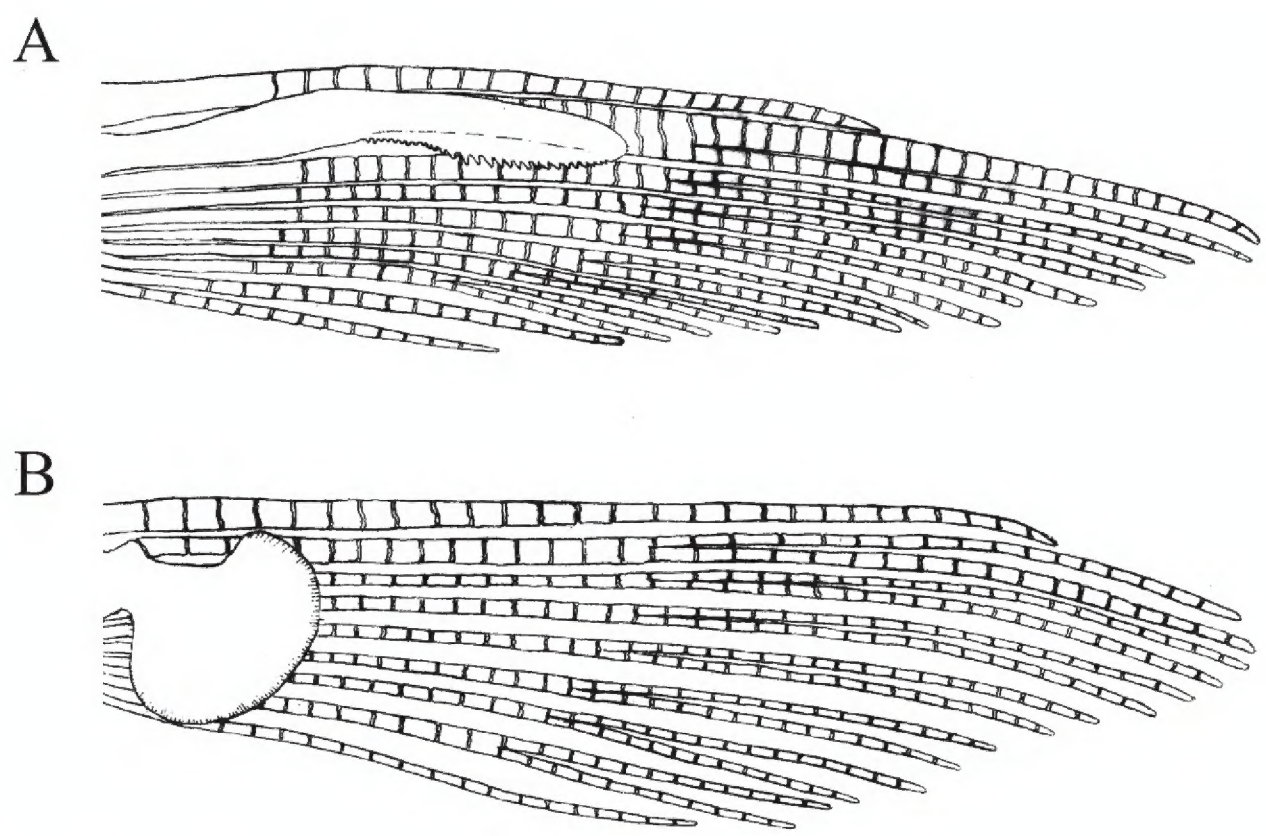

Figure 5. Lamina circularis of *Cobitis beijingensis* sp. nov. (**A**) and *C. lutheri* (**B**).

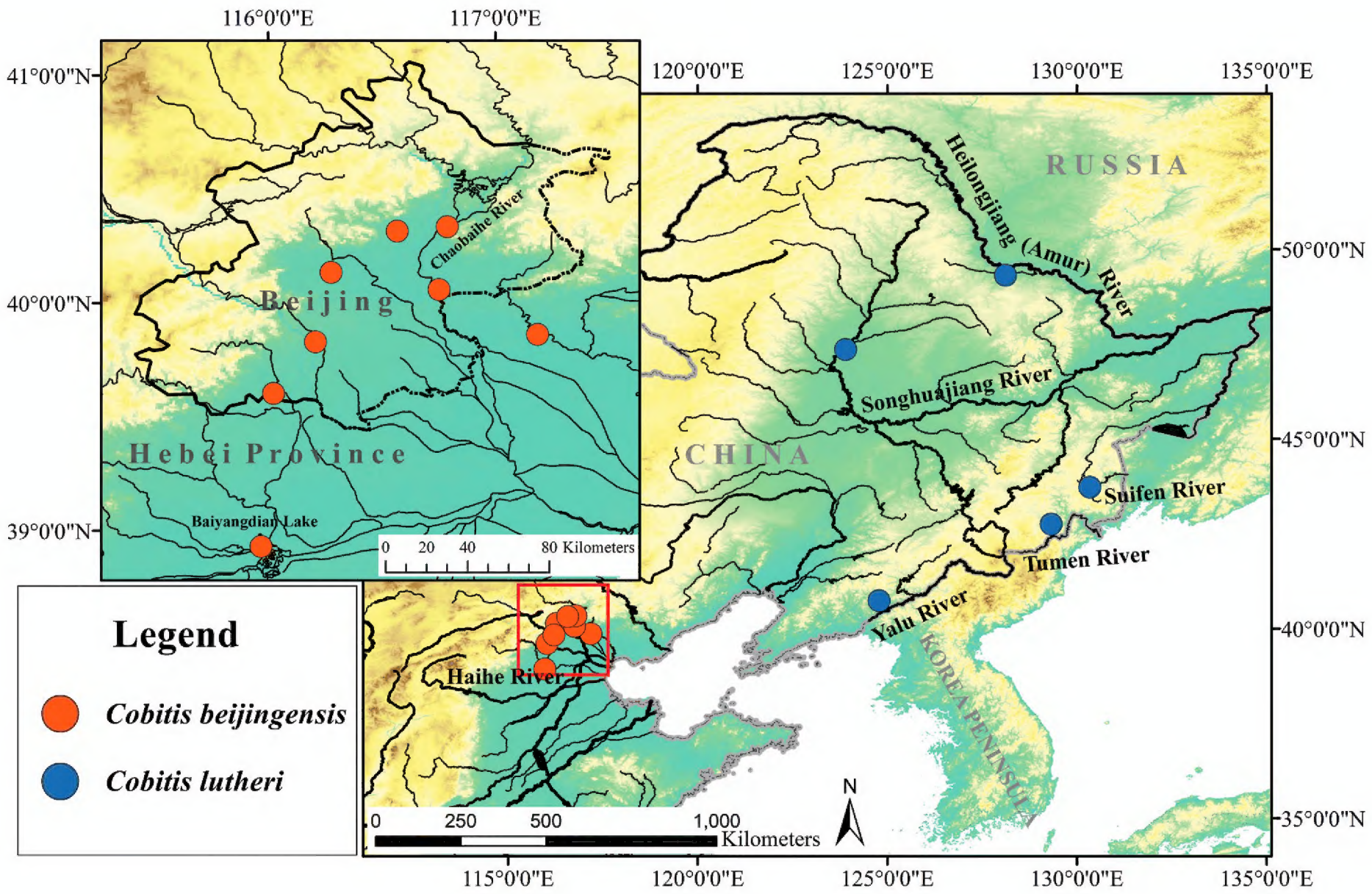

Figure 6. Distribution of *Cobitis beijingensis* sp. nov. and *C. lutheri*; the zoom-in map shows detailed distribution (i.e., specimen locality records) of the new species in Beijing and the adjacent area.

Etymology. The species name "*beijingensis*" was referred to the type locality of this species, Beijing, the capital city of China. The Chinese name for the new species is "北京花鳅".

Genetic comparisons. A total of 24 haplotypes from 30 *Cobitis* individuals were included in the analyses. The molecular phylogenetic results (BI and ML trees in Fig. 7) based on mitochondrial Cyt *b* sequences show that the new species *C. beijingensis* forms a monophyletic group itself, which is sisters to the group consists of *C. lutheri*, *C. tetralineata*, *C. nalbanti*, and *C. hankugensis*. The interspecific genetic distances between *C. beijingensis* and four congeners, *C. lutheri*, *C. tetralineata*, *C. nalbanti*, and *C. hankugensis*, are 13.1%, 8.0%, 12.5%, and 8.3%, respec-

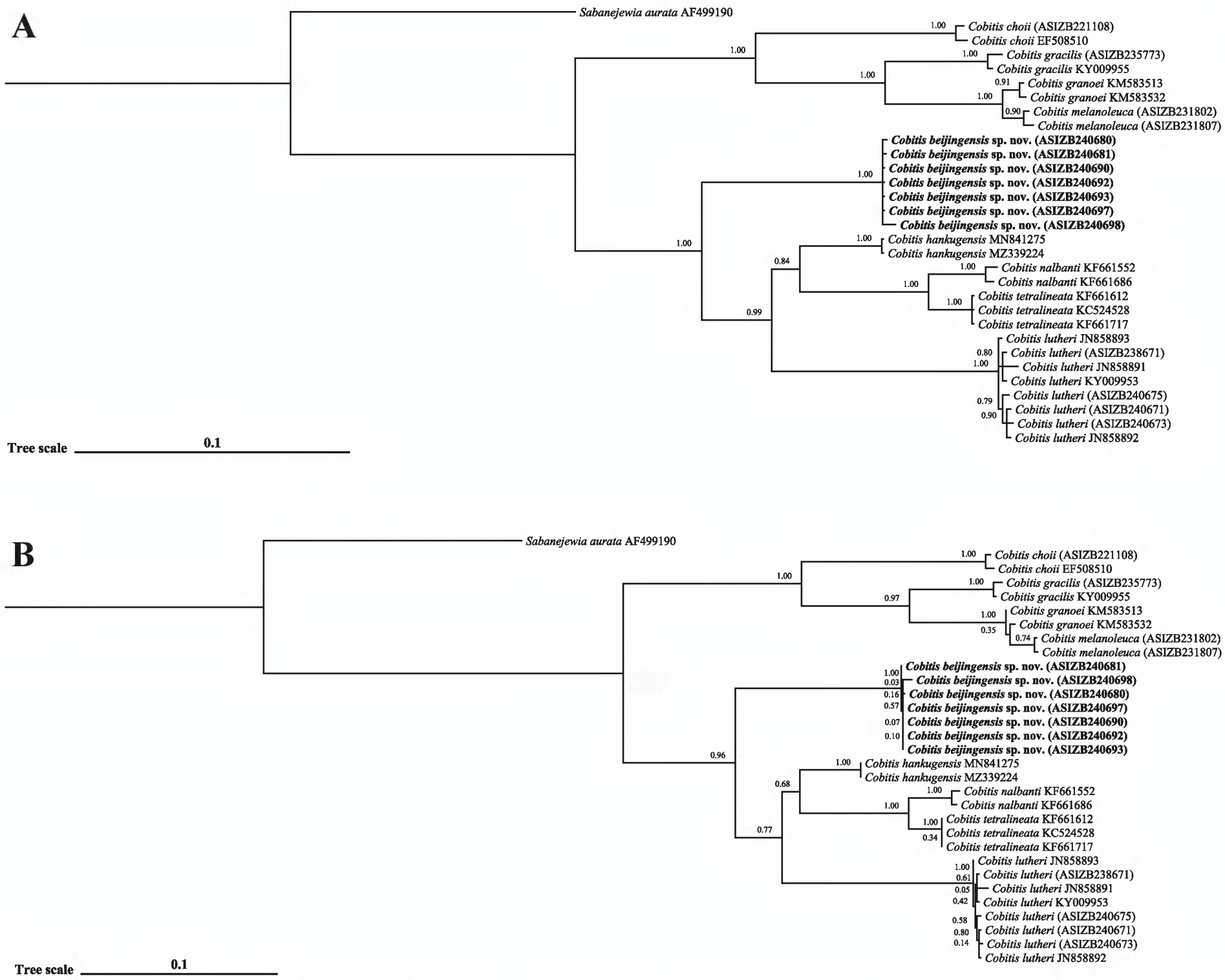

Figure 7. Molecular phylogenetic trees of *Cobitis beijingensis* sp. nov. and other phylogenetically close related congeners based on Cyt *b* sequence (1140 bp). **A.** Bayesian Inference method; **B.** Maximum Likelihood method.

tively (Table 3). The intraspecific genetic distances in *C. beijingensis*, *C. lutheri*, *C. tetralineata*, *C. nalbanti*, and *C. hankugensis* are 0.2%, 0.5%, 0.0%, 0.6%, and 0.0%, respectively (Table 3), which are much lower than the interspecific genetic distances. The molecular phylogenetic results support *C. beijingensis* to be a distinct species.

Discussion

The new species can be distinguished from the majority of the congeners in southern China by having elongated and serrated lamina circularis, except for *Cobitis microcephala* Chen & Chen, 2011. However, *C. beijingensis* sp. nov. can be distinguished from it by having a different Gambetta pattern (L_2 does not exist or is fused with L_1 vs. L_2 possessing numerous irregular dots; Fig. 8A). Amongst the nine *Cobitis* species distributed in northern China, eastern Russia, and the Korea Peninsula (Kim et al. 2003), *Cobitis beijingensis* can be distinguished from *C. melanoleuca*, *C. granoei*, and *C. gracilis* by having a posteriorly positioned pelvic-fin insertion (vs. pelvic fin inserted directly below the dorsal-fin insertion). For the rest six valid species, the new species can be further distinguished from *C. lutheri*; *C. tetralineata* Kim, Park & Nalbant, 1999; *C. pacifica* Kim, Park & Nalbant, 1999; *C. hankugensis* Kim, Park, Son & Nalbant, 2003; and *C. nalbanti* Vasil'eva, Kim, Vasil'ev, Ko & Won, 2016 by having different shapes of lamina circularis (elongated and serrated vs. rounded or kidney-shaped). For the rest one species, *C. choii*, the new species has a similar lamina circularis with it. However, the new species can still be distinguished from *C. choii* by having a different dorsal profile posterior to dorsal-fin base (horizontally extending to caudal-fin base vs. drop to caudal-fin base) and a different Gambetta's pattern (L_3 and L_4 clearly separated and linear vs. L_3 and L_4 undistinguishable and blurred; Fig. 8B).

Cobitis beijingensis is morphologically similar to *C. lutheri*, which has distributions in northern China, Russia, and North Korea (Chen and Chen 2016; Vasil'eva et al. 2016; Zhang and Zhao 2016; Antonov et al. 2019). Both species have an abbreviated and sturdy body, pelvic-fin insertion posteriorly positioned, and show a linear-shaped Gambetta's pattern in males. Despite the morphological difference between the two species mentioned above,

Figure 8. Two Chinese *Cobitis* species possessing elongated and serrated lamina circularis. **A.** *Cobitis microcephala*, ASIZB 70923, male, 49.7 mm SL; **B.** *Cobitis choii*, ASIZB 221108, male, 57.0 mm SL.

some other detailed characters can be used to distinguish them (Table 2). *Cobitis beijingensis* has more posteriorly inserted pelvic fins (inserted below 3rd–5th branched dorsal-fin rays vs. inserted below 1st or 2nd branched dorsal-fin rays), more prepelvic myotomes (14–18 vs. 11–13), and fewer vertebrae (4+36–38, mode 37 vs. 4+38–41, mode 39). The two species are distributed allopatrically (Fig. 6); *C. beijingensis* is known to be distributed in the Haihe River basin, while *C. lutheri* is distributed in the Heilongjiang (Amur) River basin, the Suifen River basin, the Tumen River basin, and the Yalu River basin.

Although *Cobitis melanoleuca* and *C. beijingensis* are all distributed in Beijing, they seem to inhabit different habitats. The former is commonly seen in the mountainous rivers where the water bodies have a certain flow velocity, usually sandy bottomed with pebbles and gravels and fewer aquatic plants. The new species, however, exists in the plain rivers or lakes where the water slows or stills, usually with plenty of aquatic plants as described above. There are several lots of specimens collected in the 1930s from different localities in Beijing, most of which were located in the plain region (Fig. 6). The Baiyangdian Lake system in Hebei Province near Beijing was also a historical distribution of this species. The abundance in historic specimens indicated a good population status in the past. However, with the development of the city, vast plains were occupied by urbanization, and the habitat of *C. beijingensis* shrank significantly. Now, the newly captured specimens are only restricted to the Chaobaihe River system in Beijing.

Comparative materials

***Cobitis melanoleuca*:** Twelve specimens. ASIZB 170312–4, 170322, 170324, 170326, 170332–3, 170339–40, 1 male, 9 female, 60.4–83.9 mm SL; Xisiduhe Village, Jiuduhe Township, Huairou District, Beijing, from the Huaijiuhe River; 1 June 1974. ASIZB 231802, 231807, 2 females; Yiduhe Village, Qiaozi Township, Huairou District, Beijing, from the Huaijiuhe River (40.34711746°N, 116.51172255°E, 91 m a. s. l.); collected by Chen Tian and Dong Sheng; 21 April 2022.

***Cobitis lutheri*:** Thirty-eight specimens. ASIZB 240646–53, 240669–79, 9 males, 10 females, 51.9–87.2 mm SL; Xinfeng Village, Luozigou Township, Wangqing County, Yanbian Korean Autonomous Prefecture, Jilin Province, from the Suifen River (43.7290824°N, 130.3484149°E, 383 m a.s.l.); collected by Zhixian Sun, Chen Tian, Yutian Fang and Dong Sheng; 27 August 2023. ASIZB 240654–68, 10 males, 5 females, 48.4–72.4 mm SL; Xunke County, Heihe City, Heilongjiang Province, from the Zhanhe River; bought by Zhixian Sun from the local fishermen; 23 May 2022. ASIZB 236417, male, 54.7 mm SL; Dixiongshan Township, Fengcheng City, Dandong City, Liaoning Province, from the Caohe River (40.79788235°N, 124.08775726°E, 156 m a. s. l.); collected by Chen Tian and Bo Li; 19 August 2022. ASIZB 238671, Zhongping Village, Dongcheng Township, Helong City, Yanbian Korean Autonomous Prefecture, Jilin Province (42.75581923°N, 129.32523131°E, 263 m a. s. l.); collected by Chen Tian and Bo Li; 6 September 2022. MCZ 32404, 4 males, 3 females, 34.4–47.7 mm SL; from the Kur River of the lower Heilongjiang (Amur) River basin, Russia; collected by Pacific Fisheries Institute, Vladivostok, U.S.S.R.

***Cobitis choii*:** One specimen. ASIZB 221108, male, 57.0 mm SL; Madigoumen village, Lijiaying Township, Xinglong County, Chengde City, Hebei Province, from the Liuhe River (40.63476888°N, 117.77879372°E, 400 m a. s. l.); collected by Xuejian Li, Zhixian Sun, and Rui Zhang; 27 September 2020.

Table 2. Counts and measurements of *Cobitis beijingensis* sp. nov. and *Cobitis lutheri*.

Counts	*Cobitis beijingensis* sp. nov.						*Cobitis lutheri*			
	Holotype	All examined specimens includes types (n = 46)					All examined specimens (n = 34)			
		Mode and Range		n			Mode and Range		n	
Branched dorsal-fin rays	7	7 (6–7)		40			7 (7)		34	
Branched anal-fin rays	5	5 (5)		40			5 (5)		34	
Branched pectoral-fin rays	8	8 (7–9)		40			8 (7–8)		34	
Branched pelvic-fin rays	6	6 (5–7)		40			6 (5–6)		34	
Prepelvic myotomes	15	15 (14–18)		39			12 (11–13)		34	
Vertebrae	4+37	4+37 (36–38)		11			4+39 (38–41)		8	
Measurements	Male	Male (n = 11)		Female (n = 15)			Male (n = 19)		Female (n = 15)	
		Mean and Range	SD	Mean and Range	SD		Mean and Range	SD	Mean and Range	SD
Standard length (mm)	55.0	55.0 (48.8–65.7)	–	64.1 (57.1–71.1)	–		54.8 (48.4–62.8)	–	70.1 (53.8–87.3)	–
In percentage of SL %										
Body depth	17.1	16.7 (12.6–19.8)	2.0	16.4 (13.3–19.3)	1.7		16.5 (14.2–18.8)	1.2	16.5 (14.7–17.9)	1.0
Head length	21.8	21.6 (19.7–25.0)	1.5	20.3 (19.0–21.4)	0.7		21.9 (20.2–23.9)	1.2	21.3 (19.4–23.5)	1.2
Dorsal-fin length	19.9	18.5 (14.8–20.8)	1.9	17.3 (15.7–19.3)	1.1		18.6 (16.3–20.8)	1.4	17.0 (14.5–18.6)	1.0
Dorsal-fin base length	10.5	10.8 (8.0–12.8)	1.4	11.4 (8.3–13.5)	1.2		10.5 (8.6–13.3)	1.1	10.5 (9.4–12.6)	0.9
Pectoral-fin length	19.4	18.4 (14.7–21.2)	1.9	13.6 (12.1–16.3)	1.2		17.1 (14.6–20.0)	1.4	13.0 (10.7–15.7)	1.0
Pectoral-fin base length	4.5	4.1 (2.6–5.3)	0.9	3.1 (2.5–3.9)	0.3		4.2 (3.5–5.0)	0.4	3.4 (2.8–3.9)	0.3
Pelvic-fin length	15.6	14.4 (12.0–16.1)	1.4	11.5 (10.1–12.7)	0.8		13.6 (11.1–15.1)	1.1	11.3 (9.6–13.1)	1.0
Pelvic-fin base length	3.8	3.5 (2.6–4.9)	0.7	3.1 (2.5–3.9)	0.4		3.4 (2.9–4.3)	0.3	3.1 (2.8–3.9)	0.3
Anal-fin length	14.7	14.0 (13.0–15.1)	0.8	12.9 (11.2–14.6)	1.0		15.3 (11.5–15.1)	1.4	13.4 (12.8–14.6)	0.6
Anal-fin base length	7.2	7.3 (5.3–8.5)	0.9	7.7 (5.9–8.7)	0.6		8.2 (6.5–10.3)	1.0	8.0 (6.9–9.7)	0.8
Pre-dorsal length	58.3	56.1 (41.3–61.1)	5.4	56.9 (52.9–61.6)	2.1		54.7 (52.4–56.8)	1.5	54.9 (52.4–58.4)	1.7
Post-dorsal length	44.2	45.6 (41.3–56.5)	4.0	46.6 (42.6–50.5)	2.1		47.2 (43.7–49.5)	1.9	47.8 (43.1–50.6)	1.8
Pre-pectoral length	23.0	23.7 (21.8–26.0)	1.4	21.9 (21.0–23.8)	0.9		24.1 (20.5–27.5)	2.1	22.0 (19.5–24.2)	1.6
Pre-pelvic length	60.5	61.3 (58.1–65.1)	2.3	58.8 (53.5–63.1)	2.5		53.7 (50.2–58.1)	2.2	53.5 (49.7–55.9)	1.6
Pre-anal length	85.1	85.0 (81.0–89.8)	3.1	83.7 (80.0–88.3)	2.2		81.8 (77.1–86.1)	2.5	81.4 (78.6–84.8)	2.0
Caudal peduncle length	8.0	10.1 (8.0–12.7)	1.4	10.5 (8.6–13.5))	1.5		11.1 (8.9–13.1)	1.3	10.8 (9.5–13.6)	1.1
Caudal peduncle depth	10.4	9.9 (8.0–11.3)	1.0	9.3 (8.6–10.0)	0.4		9.2 (8.0–11.0)	0.7	9.0 (7.6–10.0)	0.7
Head length (mm)	12.0	11.8 (11.0–12.9)	–	19.6 (12.6–26.6)	–		12.0 (10.6–13.6)	–	14.9 (12.2–17.7)	–
In percentage of HL %										
Head depth	62.0	61.7 (56.5–66.8)	3.3	62.2 (57.2–68.3)	3.2		62.1 (57.1–71.9)	3.3	61.1 (54.7–69.1)	4.0
Head width	40.5	43.1 (35.2–52.7)	6.0	44.0 (37.1–51.7)	3.8		42.5 (35.8–49.5)	4.3	45.6 (37.7–56.1)	5.2
Eye diameter	15.8	16.8 (14.2–20.3)	2.2	13.3 (11.2–16.7)	1.5		17.6 (14.0–22.2)	2.4	15.3 (12.0–17.9)	1.9
Interorbital width	27.9	24.2 (18.3–28.1)	3.8	23.1 (19.9–27.8)	1.9		21.5 (16.6–26.8)	2.6	22.2 (15.9–27.5)	3.2
Snout length	43.9	43.2 (36.9–49.3)	3.4	46.9 (41.7–50.1)	2.2		39.6 (35.6–45.4)	2.6	42.7 (37.7–48.1)	3.2

Table 3. Genetic distances of the Cyt *b* gene computed by MEGA 6.0 amongst nine analyzed species of *Cobitis*, *Sabanejewia aurata* was used as the outgroup.

		Intraspecific	1	2	3	4	5	6	7	8	9
1	*C. beijingensis* sp. nov.	0.002									
2	*C. lutheri*	0.005	0.131								
3	*C. nalbanti*	0.006	0.123	0.125							
4	*C. hankugensis*	0	0.100	0.098	0.083						
5	*C. tetralineata*	0	0.118	0.121	0.039	0.080					
6	*C. choii*	0.006	0.157	0.177	0.172	0.175	0.161				
7	*C. melanoleuca*	0.003	0.171	0.176	0.164	0.164	0.153	0.128			
8	*C. gracilis*	0.006	0.158	0.171	0.161	0.166	0.155	0.124	0.077		
9	*C. granoei*	0.004	0.175	0.181	0.162	0.165	0.156	0.130	0.015	0.076	
10	*S. aurata* (Outgroup)	NA	0.195	0.198	0.201	0.188	0.191	0.209	0.191	0.194	0.198

***Cobitis gracilis*:** Eleven specimens. ASIZB 235546–7, 2 females, 73.0–73.4 mm SL; Maxian Village, Maxian Township, Ji'an City, Tonghua City, Jilin Province, from the Yalu River (41.09436161°N, 126.14905929°E, 164 m a. s. l.); collected by Chen Tian and Bo Li, 27 August 2022. ASIZB 237892–3, 2 females, 61.9–62.8 mm SL; Sixin Village, Toudao Township, Ji'an City, Tonghua City, Jilin Province, from the Weishahe River (41.52903913°N, 125.87755765°E, 346 m a. s. l.); collected by Chen Tian and Bo Li; 28 August 2022. ASIZB 236071–4, 4 females, 53.5–76.3 mm SL; Xiaoduanshugou region, Zhenjiang Township, Kuandian Manchu Autonomous County, Dandong City, Liaoning Province, from the Yalu River (40.72689998°N, 125.44716976°E, 98 m a. s. l.); collected by Chen Tian and Bo Li; 25 August 2022. ASIZB 202947–8, 2 females, 81.9–86.3 mm SL; Kuandian local market, Kuandian Manchu Autonomous County, Dandong City, Liaoning Province; collected by Yahui Zhao; 25 September 2014. ASIZB 235773, Waichagou Village, Liangshui Korean Township, Ji'an City, Tonghua City, Jilin Province (40.92939599°N, 125.77891018°E, 129 m a. s. l.); collected by Chen Tian and Bo Li, 27 August 2022.

***Cobitis granoei*:** Ten specimens. ASIZB 199090, female, 77.1 mm SL; Ertai hydrological station, Saertuohai Township, Qinghe County, Altay, Xinjiang Uygur Autonomous Region, from the Ulungur River; collected by Chunguang Zhang; 4 September 2013. ASIZB 199498, female, 76.3 mm SL; Beitun Bridge, Beitun City, Altay, Xinjiang Uygur Autonomous Region, from the Irtyish River; collected by Chunguang Zhang. ASIZB 236765, female, 82.5 mm SL; Kalabulegen Township, Fuyun County, Altay, Xinjiang Uygur Autonomous Region, from the Ulungur River (46.40607955°N, 88.68724548°E, 690 m a. s. l.); collected by Yutian Fang and Dong Sheng; 5 July 2023. ASIZB 236677–8, 2 females, 72.2–76.0 mm SL; Fuhai Township, Fuhai County, Altay, Xinjiang Uygur Autonomous Region, from the Ulungur River (47.0968093°N, 87.4988138°E, 498 m a. s. l.); collected by Yutian Fang and Dong Sheng; 4 July 2023. ASIZB 236752–5, 236759, 2 males, 3 females, 61.0–82.3 mm SL; Dure Township, Fuyun County, Altay, Xinjiang Uygur Autonomous Region, from the Ulungur River (46.53820769°N, 88.52381182°E, 661 m a. s. l.); collected by Yutian Fang and Dong Sheng; 5 July 2023.

***Cobitis microcephala*:** Two specimens. ASIZB 70922–3, 1 male, 1 female, 49.7–65.2 mm SL; Napeng Township, Qinnan District, Qinzhou City, Guangxi Zhuang Autonomous Region, from the Dafengjiang River; collected by Chunguang Zhang and Yahui Zhao; 25 April 1999.

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Author contributions

Sun ZX contributed to the field collection, photographing, illustrating, specimen examining, data analysis, and drafting of the manuscript. Li XY contributed to specimens examining. Li XJ, Hao JY, and Sheng D contributed to field collection. Zhao YH was responsible for review and editing.

Data availability

All of the data that support the findings of this study are available in the main text.

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